# REMARKS

In paragraph 1 of the Office Action, the Examiner has noted that no specific reference to any prior nonprovisional application was included in the first of the specification of this continuation application. Applicants are submitting a Petition to Accept an Unintentionally Delayed Claim Under 35 U.S.C. §120 contemporaneously with this Response and Amendment. Therefore, Applicants are requesting entry of the attached amendment to the specification (containing the claim for priority) should the Petition be granted. Please place this new paragraph directly after the title on the first page of the specification. Applicants thank the Examiner for bringing this unintentional mistake to their attention so that it can be rectified.

In paragraph 2 of the Office Action, the Examiner rejects claim 26, alleging that the claim contains new matter in violation of 35 USC §132. The Examiner states that the specification as originally filed does not teach or describe solutions containing dideoxynucleotides as covered by claim 26. Further, in paragraphs 3 and 4 of the Office Action, the Examiner rejects claim 26 under 35 USC §112, first paragraph. The Examiner states that the specification fails to define or provide support for solutions containing dideoxynucleotides as newly claimed.

Applicants respectfully disagree with both of these assertions. The second paragraph, page 1 of the specification states (in part):

> Nucleoside triphosphates (NTP) such as ribonucleoside, deoxynucleoside and dideoxynucleoside triphosphates have a variety of uses in the field of biochemistry and molecular biology.

Further, page 3 of the specification states:

> Hence the object of the present invention was to provide a stabilized aqueous solution containing NTPs without the addition of any stabilizers. . . . The object was achieved according to the invention by the aqueous NTP solutions having a pH value of above approximately 7.5. These nucleotide triphosphates include ribonucleoside triphosphates, deoxynucleotide and dideoxynucleotide triphosphates . . ..

These statements, taken together or separately, clearly demonstrate that the Applicants specifically defined NTPs as including dideoxynucleotides, and that the Applicants understood that the inventive means for stabilizing NTPs disclosed in the specification applies to dideoxynucleotides. Thus, the matter claimed in claim 26 is not new matter, and it is fully supported by the specification. Applicants respectfully request withdrawal of the rejections made in paragraphs 2 through 4 of the Office Action.

In paragraphs 5 and 6 of the Office Action, the Examiner rejects claims 15 and 17-20 as obvious over the Promega catalog in view of Perkin Elmer Cetus. It is important to note that neither of these references (nor any other reference cited in the Office Action) suggest that a pH above 7.5 is advantageous for increasing the stability of NTPs in aqueous solution in the absence of stabilizing substances. Indeed, it is the present specification that provides the first teaching that such elevation of pH results in unexpected increases in stability.

Attached to the present Response and Amendment as Exhibit A are the results of experiments demonstrating the unexpected result obtained by the present invention. In this experiment, PCR was performed with the NTPs of the present invention (pH= 8.3, see gel marked A) and also with the NTPs of three commercial suppliers (see gels marked B, C and D. It should be noted that one product tested had a pH = 7.5, similar to the cited Promega product. NTPs produced according to the invention show a much better yield in PCR product at high template concentration and amplify the desired product at lower target concentration. As you can see from these results, there is a clear dependency of the PCR performance resulting from the increased stability of the NTPs when the pH is greater than 7.5.

These results clearly demonstrate the unexpected improvement made by changing the pH of the aqueous solution, thus increasing the stability of the NTPs. The NTPs provided by other suppliers simply do not demonstrate the stability of the NTPs of the present invention. While changing the pH might appear to be a minor change, it is clear that this change causes a major impact on stability. Further, none of the other suppliers of prior art NTPs realized this advantage, even if it is an uncomplicated solution to the problem. This is demonstrated by the simple fact that none of the NTP suppliers use solutions having a pH of greater than 7.5. This begs the question: if the solution to the stability problems was so obvious, why did the other commercial practitioners fail to appreciate the solution and sell their products at a higher pH? The answer to this is also simple. Those suppliers did not sell at higher pH values because the answer was not obvious to them.

The present invention provides an unexpected advantage over the state of the art; further, no combination of references suggests the improvement claimed, and there is no motivation to combine these references demonstrated. None of the references cited provide a teaching that a pH increase leads to more stability of the NTP solution. Even a pH=7.5 solution does not provide the stability exhibited by the present invention. For these reasons, Applicants respectfully request withdrawal of the rejection.

In paragraph 7, the Examiner rejects claims 16 and 26 as obvious over the Promega catalog in view of the Gibco BRL catalog. According to the Examiner, both claims 16 and 26 relate to modified NTPs, which are taught by the Gibco catalog, and when these known modified NTPs are combined with the teaching of the Promega catalog, the invention is obvious. Applicants respectfully disagree.

As stated above, the present invention is not suggested by any cited reference, either alone or in combination. None of the references suggests the unexpected increase in stability that occurs at pH levels higher than 7.5. This unexpected increase in stability is demonstrated throughout the specification and by the additional experimental data described above. As stated above, Applicants assert that it was not obvious that the stability of NTPs would increase when the pH is raised above 7.5. It is no more obvious to apply this improvement to modified NTPs (which Applicants readily admit were known in the art) than it was to apply this improvement to unmodified NTPs. For this reason, and because the modification was not taught or suggested by any of the cited references either alone or in combination, Applicants respectfully request withdrawal of the rejection.

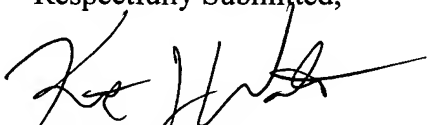
The Examiner also rejected claims 21-25 under 35 USC §103(a) as obvious over Promega catalog in view of Perkin Elmer Cetus. Applicants respectfully disagree.

These claims relate to various molecular biological methods employing the stabilized aqueous NTP solution of the present invention. As demonstrated above, the present invention provides an unexpected increase in the stability of NTP preparations. This solution to stability problems was clearly unappreciated by those practicing the state of the art; otherwise, they simply would have offered products with a higher pH to solve the problem. Because the aqueous NTP solution taught in the present specification was novel and non-obvious, the use of such stabilized NTP solutions in the various molecular biological methods is also non-obvious. Without the present improved aqueous solution, the improvement in the methods employing the solution is simply not possible.

Again, it is also important to point out that none of the cited references, either alone or in combination, suggest the use of an NTP solution having an elevated pH level as a possible means to improve such molecular biological methods. For these reasons, Applicants respectfully request withdrawal of this rejection.

Applicants believe that the claims are in condition for allowance. Applicants respectfully request that the amendment to the specification be entered, and that all rejections be withdrawn.

Respectfully Submitted,



9/19/03

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Results of Additional Experiments – Exhibit A

The performance of the dNTPs in PCR was tested in the amplification of a 4.8 kb fragment of the human tPA gene. For the amplification the Expand High Fidelity PCR System (Roche Applied Science, Cat. No. 1732641) was used according to the instructions.

Reactions were performed in 50 µl volume with different amounts of human genomic DNA (50, 10, 1 ng), 200 µM dNTP, 220 ng of each primer. The primers tPA 7 forward (5′- GGA AGT ACA GCT CAG AGT TCT GCA GCA CCC CTG C-3′) and tPA 10 reverse (5′-GAT GCG AAA CTG AGG CTG GCT GTA CTG TCT C-3′) were used.

Cycle programm:

| | |
|---|---|
| 1x | 94°C, 2 min. |
| 10x | 94°C, 10 sec. |
| | 62°C, 30 sec. |
| | 68°C, 4 min. |
| 20x | 94°C, 10 sec. |
| | 62°C, 30 sec. |
| | 68°C, 4 min., plus cycle elongation of 20 sec./cycle |
| 1x | 68°C, 7 min. |

The PCR products were analysed on a 1% agarose gel containing ethidium bromide (see attached gels). The results show that after incubation for 13 weeks at 35 °C the dNTPs according to the present invention showed a superior performance with respect to yield and sensitivity.

Legend:

Amplification of a 4.8 kb fragment of human tPA gene.
A: PCR performed with dNTPs according to the present invention
B: PCR performed with dNTPs of supplier A (pH 7.5)
C: PCR performed with dNTPs of supplier B (pH 7.0)
D: PCR performed with dNTPs of supplier C (pH 7.0)

Lanes 1: PCR performed with 50 ng template DNA
Lanes 2: PCR performed with 10 ng template DNA
Lanes 3: PCR performed with 1 ng template DNA
Lanes 4: negative control (without template DNA)

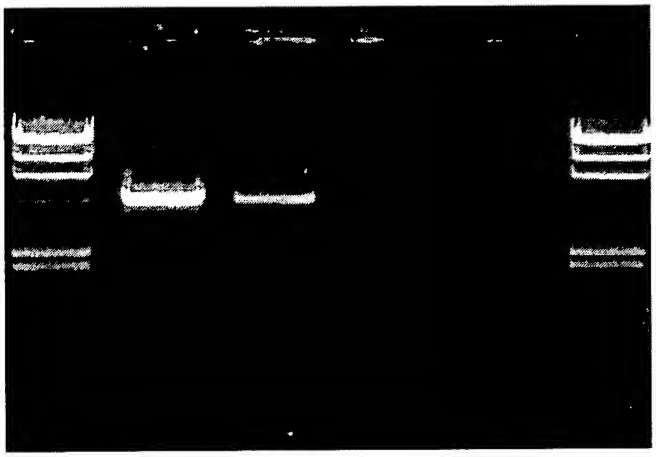
Results:

A clear dependency of the PCR-performance on the pH of the samples is observed. d-NTPs produced according to the present invention show a much better yield in PCR product at high template concentration (50 ng human DNA) and amplify the desired product at lower target concentration (10 and 1 ng).

Thus nucleotides produced according to the present invention have a much better performance in PCR assays than porducts produced according to state of the art.

A:

LS II 1 2 3 4 LS II



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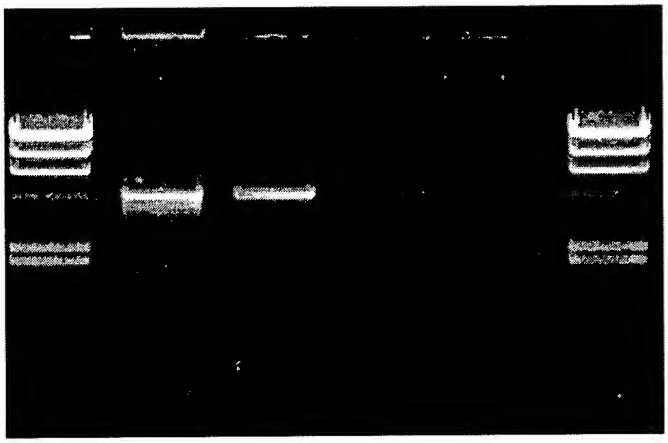
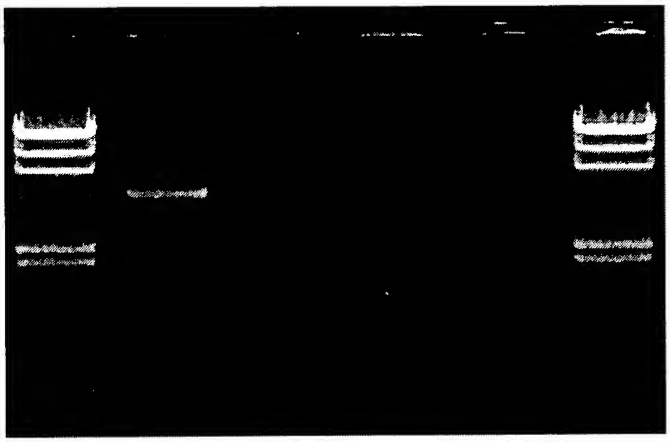
LS II 1 2 3 4 LS II



C:

LS II 1 2 3 4 LS II



D:

LS II 1 2 3 4 LS II